Figures and Tables Legends

Table 1. K-means clustering of ld-MaS steatotic relevant image features

The following morphological features: 1. radius, 2. intensity, 3. sphericity, 4. convexity, 5. aspect ratio, 6. LD-nucleus adjacency, as well as their combinations were evaluated using mean silhouette score *s* for their ability to classify ld-MaS and sd-MaS into two distinct groups. The silhouette score is a measure of cluster strength, where *s* values approaching 1 reflect high inter-cluster distance and low intra-cluster distance; thus indicate improved separation between the two groups.

Supplementary Table 1. Donor relevant clinical data

Relevant clinical data and other risk factors for the ld-MaS livers as well as occurrences of primary non function. As can be seen, livers came from a very diverse background in terms of age, cold ischemia time, body mass index, etc. In addition, some livers underwent static cold storage while others were machine perfused.
BMI- body mass index, CDC- center for disease control, HCV- hepatitis C virus, ld-MaS- large droplet macrovesicular steatosis.

Figure 1. Segmentation scheme of liver tissue and cellular structures in H&E stained liver histology images

A. H&E stained liver histology image. Bar= 50μm^2.
B. Edge function of color gradient applied to the original image.
C. Seed-points for potential steatotic droplets and nuclei were automatically initialized based on light and dark intensity contrast from the smoothed color image. These seed-points were used in the ACM.
D. ACM is utilized to refine the optimal surface area boundary by fitting each object to a model of LD or nucleus based on the following characteristics: strong edges at the boundaries, circular morphologies, smooth contours and homogeneous internal features.
E. Final segmented image indicates LDs, cell nuclei and morphological features obtained from the image.

Figure 2. Comparison of image analysis methods reporting MaS score based on total number of LDs with the pathologists' ld-MaS percentage score.

A. Steatosis percentage, as estimated by image analysis methods not separating ld-MaS from sd-MaS, compared with the average ld-MaS percentage reported by two trained pathologists from two different centers for 9 patients (N=6 images per patient were analyzed; standard error is shown).
B. Log_2 fold difference from pathologists' assessment for each method. Smaller value indicates less multiplicative deviation from the pathologists' assessment. N=9 patients; standard error is shown.
C. Fractional deviation for each method. Smaller fractional deviation indicates less additive deviation from the pathologists' assessment. N=9 patients; standard error is shown.
D. Linear regression fit (R^2) of each method relative to pathologist's ld-MaS percentage estimate. N=9 patients.

Figure 3. Comparison of image analysis methods reporting ld-MaS score based on total number of ld-MaS LDs with the pathologists' ld-MaS percentage score.

A. Ld-MaS percentage, as estimated by image analysis methods separating ld-MaS from sd-MaS, compared with the average ld-MaS percentage reported by two trained pathologists from two different centers for 9 patients (N=6 images per patient were analyzed; standard error is shown).
B. Log_2 fold difference for each method. Smaller value indicates less multiplicative deviation from the pathologists' assessment. N=9 patients; standard error is shown.
C. Fractional deviation for each method. Smaller fractional deviation indicates less additive deviation from the pathologists' assessment.
D. Linear regression fit (R^2) of each method to pathologist's ld-MaS percentage estimate.

Figure 4. Sensitivity and specificity of three image analysis methods to detect ld-MaS in H&E stained human liver tissue sections.

A. Ld-MaS LDs' sensitivity and specificity percentage of three image analysis methods: 1. 176um^2 CSSA cutoff, 2. 350 um^2 CSSA cutoff and 3. Two-level decision tree. LDs in 8 H&E stained liver histology images from 5 different patients were analyzed. Standard error is shown.
B. Three versions of the same H&E stained human liver histology image: 1. without segmentation, 2. with LD segmentation based on the 350um^2 CSSA cutoff for ld-MaS, and 3. with LD segmentation based on the decision tree method. Bar=20um^2. Black arrows point to ld-MaS LDs where their CSSA is indicated as well. White arrows indicates nuclei. Red and blue border segmentations indicate ld-MaS and sd-MaS LDs, respectively.

Supplementary Figure 1. Distinguishing ld-MaS from sd-MaS LDs based on LD size and ability to displace the nucleus.

A. H&E stained human liver histology image containing ld-MaS (black arrow) as well as sd-MaS (dashed arrow) LDs. The CSSA of LDs is indicated. The ld-MaS LD, but not sd-MaS one, is observed to be large enough to displace the nucleus, which requires the LDs to be adjacent to the nucleus (white arrow). Bar= 10μm^2
B. LD-nuclei adjacency (illustrated in Supplementary Figure 2) was determined in ld-MaS - and sd-MaS LDs based on a fixed CSSA cutoff for ld-MaS. 52,000 LDs were clustered into 7 groups based on their CSSA and the fraction of LDs with LD-nuclei adjacency ≥ 0.9 (criterion defining adjacent LD-nucleus) in each group was determined. Specific CSSA cutoffs and their corresponding fraction of adjacent LD-nuclei are indicated.

Supplementary Figure 2. LD-nucleus adjacency parameter.

The shortest distance between each LD's perimeter and the perimeter of its nearest nucleus was quantified and LD-nucleus adjacency was calculated as follows: LD-nucleus adjacency = Radius of LD/ Radius of LD+ Distance between LD and nucleus perimeters. Adjacency values ≥ 0.9 up to the theoretical limit of 1 were used to define LDs adjacent to the nucleus.

Supplementary Figure 3. Decision tree structure.

A decision tree algorithm was generated to distinguish between ld-MaS and sd-MaS. The decision tree was trained and optimized from the raw data using N-fold cross-validation (N=10) to yield two levels of decision making: The first level assigns sd-MaS and ld-MaS labels to LDs that have CSSA values below a low threshold or above a high threshold, respectively. The second level deals with the LDs that have CSSAs that fall between the low and high threshold, and uses LD-nucleus adjacency as a criteria to ld-MaS.

Supplementary Figure 4. Applying the decision tree method to frozen section images.

A. H&E stained frozen section human liver histology image (left) and ld-MaS/ sd-MaS segmentation using the decision tree algorithm (right). The method was successful in segmenting LDs while properly excluding empty spaces artifacts (indicated by arrows) seen on frozen sections that may have been confused with LDs.
B. H&E stained frozen section human liver histology image (left panel) with unsegmented (top) or segmented for ld-MaS/ sd-MaS using the decision tree algorithm (bottom) is compared to H&E stained paraffin embedded section human liver histology image from the same liver (right panel) unsegmented (top) or segmented using the same method (bottom). The segmentation of the frozen section was in agreement with the pathologist's evaluation and comparable to the results obtained from the paraffin embedded tissue of the same liver. Bar=50um, red segmentation= ld-MaS, blue segmentation= sd-MaS and black segmentation= nucleus.